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Decline of the Hemoglobin Adduct of 4-Aminobiphenyl during Withdrawal from Smoking[1]

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ABSTRACT

The hemoglobin adduct of the human bladder carcinogen 4-aminobiphenyl (4ABP-Hb) declined in the blood of 34 smokers enrolled in a withdrawal program, from a mean of 120 ± 7 (SE) pg/g of hemoglobin at the start to a mean of 82 ± 6 pg/g after 3 weeks and a mean of 34 ± 5 pg/g among the 15 exsmokers who had not resumed smoking after 2 months. Although 4ABP-Hb declined faster than expected under the assumption that the human erythrocyte has a life span of 120 days, it persisted much longer than cotinine. Therefore, 4ABP-Hb may complement the use of cotinine as a marker of exposure to tobacco smoke. The strength of the within-person association of 4ABP-Hb with smoking, coupled with the weakness of the between-person association (correlation coefficient, 0.33), is evidence that between-person variation in modifying factors is substantial. Study of the modifiers of 4ABP-Hb levels may help elucidate the etiology of human susceptibility to aromatic amine-induced bladder cancer.

INTRODUCTION

The aromatic amine 4ABP[4] is a potent human bladder carcinogen (1, 2) and is present in tobacco smoke (3). Its carcinogenicity is believed to derive from its *N*-hydroxylation and subsequent hydrolysis yielding an electrophilic nitrenium ion that binds with DNA and other macromolecules (4, 5). We have shown in a rat model (6, 7) that the acid-labile sulfinamide adduct, formed by reaction of *N*-hydroxy-4-aminobiphenyl with a cysteine residue of hemoglobin, reflects average exposure to 4ABP over the life span of the rat erythrocyte. Using a sensitive gas chromatography-mass spectrometry assay (8), we have also shown that blood levels of 4ABP-Hb are 2 to 10 times higher in smokers than in nonsmokers (8–10). To assess the potential of the assay as a dosimeter for epidemiological studies of 4-aminobiphenyl, we undertook a validation study involving 34 smokers enrolled in a withdrawal program. Our hypotheses were: (*a*) adduct levels would decline during withdrawal; (*b*) the rate of decline would correspond to the rate of replacement of erythrocytes after a life span of 120 days; (*c*) differences among the initial adduct levels of smokers before they began the program would be explained by differences in the number and type of cigarettes smoked; and (*d*) 4ABP-Hb would be as good a dosimeter of tobacco smoke exposure as plasma cotinine, a metabolite of nicotine now widely used as a biochemical marker of smoking.

MATERIALS AND METHODS

Volunteers were recruited from smoking withdrawal classes conducted by The Stop Smoking Clinic (Danvers, MA) at 7 suburban hospitals in Eastern Massachusetts. Their ages ranged from 23 to 78 years, with a mean of 44 years. Fifty-five % of them were women. The initial blood sample was collected from 75 subjects at the beginning of a 2-week reduction period. Tar and nicotine intake was reduced 75% during the first week and the remaining 25% during the second week, inasmuch as the program required the participants not only to smoke steadily fewer cigarettes per day but also to switch to brands with progressively lower tar and nicotine levels. The second blood sample was collected 3 weeks after the first, and the third blood sample was collected 6 to 8 weeks after the second.

Blood samples were drawn into 10-ml EDTA vacutainers and refrigerated overnight. Erythrocytes were separated from plasma, washed in saline, and frozen. Plasma was frozen and sent to the American Health Foundation, Valhalla, NY, for determination of cotinine by a modified radioimmunoassay (11, 12). A plasma cotinine level below 10 ng/ml was taken as an indicator of successful withdrawal (13). After all blood samples had been collected from a subject, the cells were thawed, processed, and assayed simultaneously to prevent confounding by any day-to-day variation in laboratory methods.

The procedures of sample preparation and analysis have been described in detail elsewhere (8). In brief, hemoglobin was isolated from RBC and purified by dialysis. The dialysate was spiked with 1 ng of an internal standard (4′-fluoro-4-aminobiphenyl) and then hydrolyzed to release free 4ABP. The amines were extracted into purified hexane and then derivatized to form the corresponding pentafluoropropionamides. These were quantified using capillary gas chromatography with detection by negative-ion chemical ionization mass spectrometry.

The precision of the assay was quantified by measuring the coefficient of variation after splitting two 100-ml blood samples into 8 and 7 aliquots and by comparing duplicate blood samples collected 15 to 48 h apart from 15 people (8).

Differences and associations were examined both graphically and statistically. Mean differences and correlation coefficients were calculated with 95% confidence intervals. Differences between the observed and expected percent declines in excess adduct as a function of time were tested by the one-sample *t* test.

The observed percent decline in excess adduct due to smoking was defined as the decline in the difference between the observed adduct level and the final adduct level attained by the exsmokers. The percent decline in the excess between the first and second blood samples was calculated, and likewise between the first and third samples, using three different estimates of the final attained level: the mean adduct level in 9 subjects who had not resumed smoking 4 months after withdrawal (33 pg/g of hemoglobin); and the upper and lower limits of the 95% confidence interval about the mean (23 and 44 pg/g of hemoglobin).

The expected percent decline in excess at time, *t*, was computed with the assumption of sudden cessation of exposure and uniform life expectance, *L*, for all erythrocytes. Under these assumptions, the decline in excess adduct is expected to be quadratic, not linear, because two processes of linear decline take place simultaneously and interact. Not only is there a linear fall in the number of cells ever exposed to the excess 4ABP from smoking but there is also a linear fall in the average cumulative exposure of the remaining exposed cells. This is because the first erythrocytes to be replaced are the oldest, the cells with the greatest cumulative exposure to aromatic amine. After the first 1% of cells are replaced, 99% of the remaining cells were ever exposed to adducts from tobacco smoke and, among them, the average excess of adduct per cell is 99% the original average. Therefore, the total excess adduct remaining in the blood is 99% × 99% = 98%. In general, the remaining fraction of the original excess of adduct equals $(1 - t/L)^2$, where t/L is time since exposure cessation, expressed as a fraction of

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[4] The abbreviations used are: 4ABP, 4-aminobiphenyl; 4ABP-Hb, 4-aminobiphenyl-hemoglobin.

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